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OVIPOSITIONAL BEHAVIOR AND LARVAL DEVELOPMENT OF *APHIDENCYRTUS APHIDIVORUS* (HYMENOPTERA: ENCYRTIDAE), AN APHID HYPERPARASITOID

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Abstract.—In most hyperparasitoids, the female wasp attacks the primary parasitoid host either while the parasitized aphid is still alive or after the mummy is formed. *Aphidencyrtus aphidivorus* (Mayr) had been known to have a "dual" ovipositional behavior. Whether the adult female wasp attacked the primary parasitoid host through a live parasitized aphid or a mummified aphid, her egg was always deposited inside the host larva and not on the surface. The egg hatched inside the host larva, whereupon the hyperparsitoid larva first fed endophagously, and then ectophagously until the primary host larva was consumed. In "choice" experiments, 82% of the female *A. aphidivorus* wasps preferred to attack the primary parasitoid larva through the mummy, while only 18% chose the live parasitized aphids for oviposition.

Aphid hyperparasitoids or secondary parasitoids can be divided into two categories based on their combined adult ovipositional and larval feeding behavior (Sullivan, 1986, 1987, 1988): (1) endophagous species in the families Charipidae (*Alloxysta, Phaenoglyphis, Lytoxysta*) and Eulophidae (*Tetrastichus*) in which the female wasp deposits her egg inside the primary parasitoid larva while it is still developing inside the live aphid (before the aphid is mummified); the egg does not hatch until the mummy is formed, at which time the hyperparasitic larva feeds internally on the primary parasitoid larval host, and (2) ectophagous species in the families Pteromalidae (*Asaphes, Pachyneuron, Coruna*) and Megaspilidae (*Dendrocerus*). In these hyperparasitoids, the female wasp deposits her egg on the surface of the primary parasitoid larva after the aphid is killed and mummified. The hyperparasitic larva then feeds externally on the primary parasitoid larval host while both are still within the mummy.

Earlier studies on the biology of *Aphidencyrtus aphidivorus* (Mayr) (Encyrtidae) by Silvestri (1909), Griswold (1929), and Maple (1947) have documented "dual" ovipositional behavior in this species. Matteson (1977) reported that female *Aphidencyrtus aphidivorus* attack and oviposit into both live, parasitized aphids and mummified aphids. Details of this dual ovipositional behavior as well as the endo- and ectophagous development of the larva are described. In addition, the present laboratory study includes results of "choice" experiments by female *A. aphidivorus* between a primary parasitoid larval host in a live aphid or in a mummy, which might indicate preferential ovipositional behavior.

MATERIALS AND METHODS

Host plant and aphid.—The broad bean, *Vicia faba* Linnaeus (Windsor variety), was used to rear the pea aphid, *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae), according to the method described by Bennett and Sullivan (1978). Plants and aphids were kept in a Percival bioclimatic chamber. The daytime regime had a photoperiod of 16 hr, a temperature of 21.1 ± 0.6°C and 75 ± 5% RH. At night the photoperiod was 8 hr of darkness, at 15.5 ± 0.6°C and 85 ± 5% RH. This bioclimatic chamber and the same environmental conditions were also used for separate, caged rearings of the primary parasitoids, hyperparasitoids, and for the "choice" experiments.

Primary parasitoid and hyperparasitoid.—*Aphidius smithi* Sharma and Subba Rao (Hymenoptera: Aphidiidae) was the primary parasitoid. Our colony originated from the Division of Biological Control Gill Tract (Albany), of the University of California at Berkeley. The secondary parasitoid or hyperparasitoid was *Aphidencyrtus aphidivorus* (Mayr) (Hymenoptera: Encyrtidae), which was reared from pea aphid mummies collected on alfalfa in Lafayette (Sussex County), New Jersey. In preparation for the photographic studies on egg deposition and larval development, we dissected a minimum of 30 live parasitized aphids and 30 mummified aphids.

"Choice" experiments.—After mating, 3 female *A. smithi* were introduced into a stinging-tube (glass cylinder 15 cm long and 3.3 cm in diameter and covered at both ends with a fine mesh organdy cloth, secured by rubber bands) containing a cut broad bean plant stem and 10–15 pea aphids always in the 4th instar. The stinging-tube was kept in the bioclimatic chamber for a 6 h ovipositional period. At the end of that time, the primary parasitoids were removed, and the aphids placed on growing bean sprouts and returned to the bioclimatic chamber. After 8 d, the aphid was killed by the primary parasitoid larva which had been feeding internally, and a mummy was formed, followed by pupation.

In the "choice" experiments, however, we started by first using one of the live, but parasitized, pea aphids that had been attacked by *A. smithi* 7 d earlier in a stinging-tube. At this stage of development, the parasitoid would be a 4th instar larva. This parasitized aphid was paired with a dead or mummified aphid (containing a 9-day old *A. smithi* prepupa or early pupa). Both were placed on a segment of broad bean leaf or stem in a Petri dish. One mated female *A. aphidivorus,* that had not been given the opportunity to oviposit previously, was put into the Petri dish equidistant between the two potential hosts (live parasitized aphid and mummy) and permitted to oviposit. The behavior of *A. aphidivorus* was observed continuously during a period of 2 hr maximum. Once the ovipositor was withdrawn from either one of the two potential hosts, the female *A. aphidivorus* was removed from the Petri dish. This might be much less than the 2 hr oviposition period, for in no case was the female permitted to oviposit into the 2nd host, nor remain beyond this maximum time limit even if she had not oviposited at all.

Once the hyperparasitoid was removed from the Petri dish, the mummy was placed in a gelatin capsule to await emergence of an adult wasp. The live aphid, however, was permitted to continue feeding on the broad bean for 48 hr or until it was killed and mummified by the *A. smithi* parasitoid larva inside. If a live aphid was not really parasitized and killed, then this particular paired replicate was discarded from the

experiment. This newly formed mummy was also placed into a separate gelatin capsule. If the primary parasitoid, *A. smithi,* emerged (approximately 4 d after mummy formation), then oviposition by *A. aphidivorus* would not have occurred, or at least hyperparasitism would not have been successful. If on the other hand, the hyperparasitoid, *A. aphidivorus,* emerged (about 21–23 d after the attack), then this indicated effective hyperparasitism of whatever primary host was chosen: either in a live aphid or in a mummy. A total of 175 paired replicates was used in these choice experiments that resulted in 175 adult emergences of *A. aphidivorus.*

RESULTS AND DISCUSSION

Oviposition into a live parasitized aphid.—After initially antennating the live parasitized aphid (usually for less than a minute), the female *A. aphidivorus* mounted the dorsum, always facing caudally (Fig. 1). With her antennae pointed downward, motionless and touching the aphid, the female inserted her ovipositor through the integument of the aphid, pushing it into the hemocoel. With up and down thrusts, she probed for the larva of the primary parasitoid, and later dissections showed that the egg is laid internally in the *A. smithi* larva. After withdrawing her ovipositor, the *A. aphidivorus* sometimes host fed on the fluid that may ooze from the puncture hole of the aphid. The process of oviposition lasted 3–5 minutes.

Oviposition into a mummy.—This is similar to the behavior observed when attacking a live parasitized aphid. There was the usual antennating by the female, and she eventually climbed onto the dorsal surface of the mummified aphid. As in the behavior described when attacking a live parasitized aphid, the antennae were pointed downward and remained motionless, with the female facing caudally (Fig. 2). She then drilled a hole through the mummy wall with her ovipositor (Fig. 3), moving her body rapidly up and down. The ovipositor was pushed into the mummy, and as later dissections have shown, the egg was again laid inside the larva of *A. smithi.* This is unusual among hyperparasitoids that attack a primary parasitoid larva within a mummy, because the egg is normally deposited on the surface of the host in the mummy (Sullivan, 1986, 1987, 1988). After oviposition, the female withdraws her ovipositor and sometimes host feeds at the drill hole. However, a "feeding tube" is not formed as has been described for the host feeding behavior at the mummy by *Asaphes lucens* (Keller and Sullivan, 1976). The duration of oviposition into the mummy took 4–8 minutes, somewhat longer than when ovipositing into a live parasitized aphid. This was probably because of the need to penetrate the hard mummy wall.

Developmental stages of Aphidencyrtus aphidivorus.—As mentioned earlier, the female always placed the egg inside the primary parasitoid larva, never on the surface, regardless of whether the host was attacked in a live aphid or in an aphid mummy. When a 9-day old *A. smithi* was attacked through the mummy, this host was a prepupa or early pupa. In this case, *A. aphidivorus* required approximately 21 days to develop from egg deposition to adult emergence. However, if *A. smithi* was attacked while the aphid was still alive, the host was a 4th instar larva and the hatching of the egg was delayed 24–48 hr until the primary parasitoid larva killed the aphid and formed the mummy. This time difference was probably due to the developmental stage of the *A. smithi* host in relation to the aphid being alive or mummified, so that


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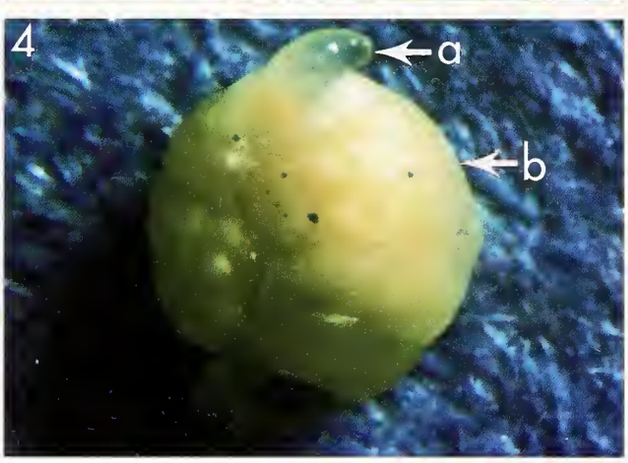
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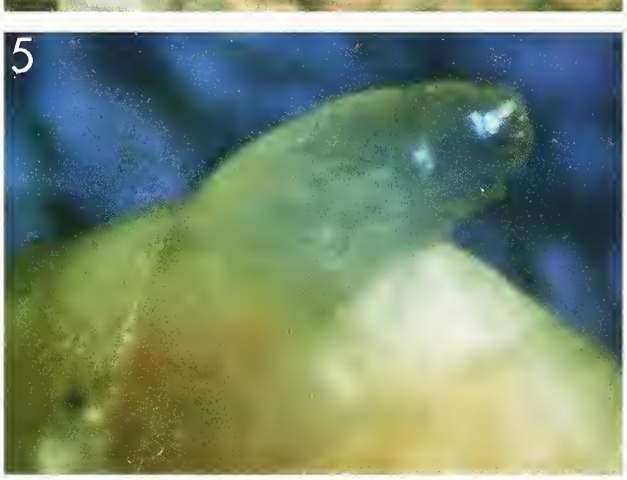
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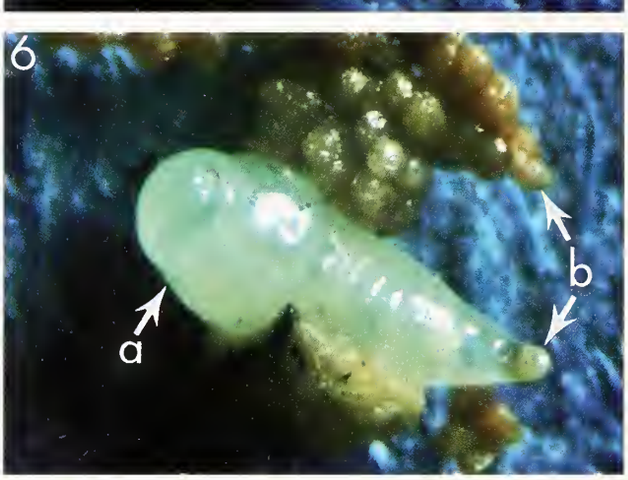
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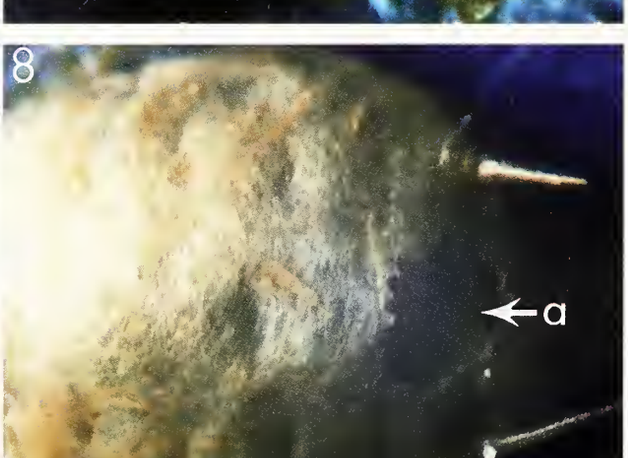
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the adult emergence of *A. aphidivorus* occurred 22–23 days after oviposition when a live parasitized aphid was attacked.

While still within the aphid mummy, the 1st and 2nd larval instars of *A. aphidivorus* fed endophagously within the primary parasitoid host, but the 3rd instar larva ate through the exoskeleton of the *A. smithi* larva and emerged from the host (Figs. 4–5). This occurs 9 d after egg deposition, and for the next 48 h, the *A. aphidivorus* larva continued to feed ectophagously until the primary parasitoid host was consumed.

When feeding ceased, the hyperparasitoid larva voided its meconium in the form of 35–40 small, sticky, yellow-orange, spherical pellets and became a prepupa (Fig. 6). These meconium pellets were easily distinguishable from the sausage-shaped, black meconium of *A. smithi.* After 48 hr, the pupa (Fig. 7) is recognizable and lasts 7–9 d, until the adult cuts its way out of the mummy by making an uneven, saw-edged exit hole (Fig. 8) in the aphid mummy, usually through the posterior dorsal surface. Then the adult *A. aphidivorus* pushed its head through the hole and pulled itself out of the mummy. This emergence occurs about 21–23 d after the original egg deposition; males usually emerged before females.

Choice experiments.—82% (N = 287) of the *A. aphidivorus* females oviposited into mummies, while only 18% (N = 63) chose the live parasitized aphids for oviposition.

When describing host selection by *A. aphidivorus,* Matteson (1977) reported that "Of the 60 hosts accepted for probing, 33 were mummies and 27 were live aphids. A total of 42 eggs were laid, 22 in *T. pallidus* larvae inside live aphids. These figures indicate no preference between hosts in live aphids and hosts in mummies for probing or oviposition." *Trioxys pallidus* (Haliday) is a primary parasite of the walnut aphid, *Chromaphis juglandicola* (Kaltenbach). Our data, on the other hand, are based on a different primary parasitoid and a different aphid species; and show a parametric statistically significant preference ($P = <0.01$) by *A. aphidivorus* for hyperparasitism of the primary parasitoid larval host in mummies (82%) over that in live aphids (18%). Perhaps this preference is elicited by some factor or combination of factors, such as visual and/or chemical stimuli, but this important aspect is beyond the scope of the present study.

One might speculate about the evolution of this "dual" ovipositional behavior of *A. aphidivorus.* Data from related species are needed to clarify whether this hyperparasitoid primitively exhibited ovipositional behavior whereby it attacked the primary parasitoid larva in a live aphid, or whether it primitively waited for the host larva to kill the aphid, form a mummy, and only then oviposit into it. At present, it is not clear which of these two types of behavior is "primitive" or "advanced."

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Figs. 1–8. 1. *Aphidencyrtus aphidivorus* ovipositing into live parasitized aphid (×10); 2. *A. aphidivorus* ovipositing into mummified aphid (×6.4); 3. *A. aphidivorus* with exserted ovipositor (a) drilling into mummified aphid (×13); 4–5. Nine-day old 3rd instar *A. aphidivorus* larva (a) emerging from *Aphidius smithi* larva (b) and beginning to feed as an ectoparasitoid (×13, ×32); 6. Ventral view of 11-day old *A. aphidivorus* prepupa (a) voiding meconium at caudal end (b) (×13); 7. Ventral view of 16-day old *A. aphidivorus* pupa with meconium (×19); 8. Saw-edged exit hole of (a) *A. aphidivorus* located on posterodorsal side of mummified aphid (×13).

Also unusual about this hyperparasitoid is that unlike other genera of secondary parasitoids that attack the primary parasitoid through the mummy and deposit the egg externally on the surface of the host, *A. aphidivorus* oviposits inside the primary host within the mummy, thus behaving more like the endophagous hyperparasitoids that attack live parasitized aphids.

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